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ORIGINAL ARTICLE

Association of *ATXN2* intermediate-length CAG repeats with amyotrophic lateral sclerosis correlates with the distributions of normal CAG repeat alleles among individual ethnic populations

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Abstract

Intermediate-length CAG repeats in *ATXN2* have been widely shown to be a risk factor for sporadic amyotrophic lateral sclerosis (SALS). To evaluate the association of *ATXN2* intermediate-length CAG repeat alleles with an increased risk of SALS, we investigated distributions of CAG repeat alleles in 394 patients with SALS and 490 control individuals in the Japanese population. In the intermediate-length repeat units of 29 or more, we identified one SALS patient with 31 repeat units and two control individuals with 30 repeat units. Thus, no significant differences in the carrier frequency of intermediate-length CAG repeat alleles were detected between patients with SALS and control individuals. When we investigated the distribution of "large normal alleles" defined as *ATXN2* CAG repeats ranging from 24 up to 33 in the Japanese population compared with those in other populations in previous studies, the frequency of large normal alleles was significantly higher in the European and North American series than in the Japanese series. Moreover, these frequencies in the Turkish, Chinese, Korean, and Brazilian (Latin American) series were also higher than that in the Japanese series. These results raise the possibility that the frequencies of large normal alleles in individual populations underlie the frequencies of ALS risk alleles in the corresponding populations.

Keywords Amyotrophic lateral sclerosis · *ATXN2* · CAG repeat expansion · Spinocerebellar ataxia type 2 · Intermediate-length repeat

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the progressive degeneration of upper and lower motor neurons. Only 5–10% of ALS cases are familial (FALS), whereas the majority of cases are sporadic (SALS) [1]. Currently, as many as 26 genes have been reported to be associated with FALS [2, 3]. In our recent study, pathogenic mutations in ALS causative genes, including *SOD1*, *FUS*, *TARDBP*, *VCP*, *ERBB4*, *HNRNPA1*, *TBK1*, and *C9ORF72*, were identified in 54% of families with FALS and 3.9% of patients with SALS in the Japanese ALS case series [4–10]. Of note, the frequency of patients carrying the repeat expansion mutations in *C9ORF72* is considerably lower in the Japanese population than in European populations [11], with the exception of the concentration of the *C9ORF72* repeat expansion in the patients with ALS in the Kii Peninsula [10]. Thus, pathogenic mutations in the causative genes for FALS account for only a small population of patients with SALS and the molecular basis of SALS largely remains unknown.

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To elucidate the molecular basis of SALS, a number of genome-wide association studies (GWAS) have been conducted, which identified ALS-susceptibility genes, including *C9ORF72*, *FGGY*, *ITPR2*, *DPP6*, *KIFAP3*, and *UNC13A*, in the populations of European ancestry [12], *SUSD2* in the Chinese population [13], and *ZNF512B* in the Japanese population [14]. With the exception of the discovery of the association of SALS with *C9ORF72* in patients of Finnish ancestry, these associated genes await further replication studies in large case series including independent populations.

In addition to susceptibility genes identified by GWAS, intermediate lengths of CAG repeats ranging from 27 to 33 in *ATXN2* have been identified to be significantly associated with ALS in North American (Caucasian origin) series [15]. Thereafter, many studies have confirmed that intermediate-length *ATXN2* alleles contribute to an increased risk of ALS in various ethnic populations, including European and North American, Turkish, Chinese (mainland and Taiwan), Korean, and Brazilian (Latin American) populations, although different definitions of intermediate-length repeat units (≥ 27, ≥ 29, or ≥ 30) have been used to investigate associations of these alleles with ALS [16–18]. CAG repeats in *ATXN2* are fully expanded (33 or more repeat units) in patients with spinocerebellar ataxia type 2 (SCA2), an autosomal dominant neurodegenerative disorder [19–21].

The association of intermediate-length *ATXN2* repeats with SALS in the Japanese population, however, remains to be elucidated. In this study, we performed an association study on a Japanese case-control series to determine whether intermediate-length *ATXN2* repeats contribute to an increased risk of ALS in the Japanese population. We further investigated whether the differences in distributions of normal *ATXN2* CAG repeat alleles among individual populations underlie the differences in associations of intermediate-length *ATXN2* repeat alleles with SALS.

Materials and methods

A total of 410 Japanese patients with SALS were recruited for clinical and molecular genetic studies. All the patients were diagnosed as having clinically definite, probable, laboratory-supported probable, or possible ALS on the basis of the El Escorial revised criteria [22]. We sought to investigate the association of intermediate-length *ATXN2* CAG repeats, in particular, with SALS cases. In addition, 490 unrelated healthy Japanese participants with no reported history of neurological diseases were included as a source of control DNAs. Genomic DNA samples were obtained from all the participants with their written informed consent, and this research was approved by the institutional review board of the University of Tokyo.

Mutational analysis of causative genes for FALS was conducted prior to this study [5]. Sixteen patients with SALS carrying pathogenic mutations in the genes for FALS and the remaining 394 patients with SALS not carrying pathogenic mutations were analyzed separately, since pathogenic mutations for FALS are considered to exert major roles in developing ALS. The remaining 394 patients with SALS were enrolled in this study. The length of CAG repeats in *ATXN2* was determined by fragment analysis as previously reported [19]. The mean (SD) age at onset of the 394 patients with SALS was 60.3 (12.7) years, and the male-to-female ratio was 1.45:1. Regarding the site of symptom onset, the proportions of subjects with initial bulbar, upper extremity, and lower extremity symptoms were 27%, 38%, and 35%, respectively. Frontotemporal dementia (FTD) was detected in 11 patients with SALS (2.8%). In the 490 healthy control individuals, the mean (SD) age at sampling was 39.4 (13.0) years, and the male-to-female ratio was 1.11:1. We defined the intermediate-length CAG repeat units of 29 or more as the ALS risk alleles, because they have been reported to be associated with ALS in a recent meta-analysis [16]. In addition, we hypothesized that differences in the distribution of normal CAG repeat alleles in individual populations may underlie differences in associations of *ATXN2* intermediate-length alleles with ALS in the corresponding populations. To explore this possibility, we arbitrary designated normal CAG repeat alleles that are larger than 23, and not pathogenic for SCA2 (≤ 33), as "large normal alleles" [16, 23] to discriminate from the intermediate-length alleles that are associated with the risk of ALS. We investigated the distribution of frequencies of CAG repeat units in these Japanese patients with SALS and healthy control individuals compared with those previously reported in other populations, including European and North American [15, 16, 24–34], Turkish [35], Chinese (mainland) [36, 37], Chinese (Taiwan) [38], Korean [17], and Brazilian (Latin American) [18] series.

The Fisher's exact test for 2×2 tables was used to test for differences in the carrier frequency of ALS risk alleles with the cutoff of ≥ 29 repeat units between patients with SALS and control individuals in the Japanese series. Based on a meta-analysis, a pooled OR was calculated using a fixed effects model (Mantel-Haenszel method). Differences in the frequencies of large normal alleles in the control individuals between the Japanese series and each of the other series populations were also analyzed by Fisher's exact test for 2×2 tables, and Bonferroni corrections were applied for six pairwise comparisons between the Japanese and the other six ethnic populations. Statistical analysis was carried out using R version 3.4.1, and EZR package, a recently developed graphical user interface for R [39]. All the statistical tests were two-sided, and an adjusted p value of less than 0.01 was considered statistically significant.

Results

In the 394 patients with ALS, 22 repeat units were the most common (94.9%) with a range from 13 to 31. The most common repeat units in the 490 controls were also 22 (95.0%), with a range from 12 to 30. In the intermediate-length CAG repeat units of 29 or more, we identified one SALS patient with 31 repeat units and two control individuals with 30 repeat units. In the additional analyses, none of the 16 patients with SALS carrying pathogenic mutations in the genes for FALS had intermediate-length repeat alleles in *ATXN2*; hence, we could not evaluate the effect of intermediate-length repeat alleles on these SALS patients. The patient with SALS carrying an intermediate-length allele of 31 repeat units was a 62-year-old male at the time of diagnosis of ALS, who developed lower extremity weakness over 1 year. Presence of concomitant dementia was not evident when the diagnosis of ALS was made, and detailed follow-up information on the clinical course including time to respirator or death was unavailable. The result indicates that there was no significant difference in the carrier frequency of intermediate-length CAG repeat alleles between patients with SALS and control individuals in the Japanese series in this study. When we used other threshold values to define intermediate-length repeat units (≥ 26, ≥ 27, ≥ 28, ≥ 29, or ≥ 30), the same results confirming the absence of association of *ATXN2* intermediate-length CAG repeat alleles with ALS were also obtained in this study.

Our finding was in striking contrast to previous studies where significant associations of intermediate-lengths CAG repeat alleles in patients with SALS have been demonstrated in European and North American, Turkish, Chinese (mainland and Taiwan), Korean, and Brazilian (Latin American) populations. We then investigated the distribution of intermediate-length CAG repeat alleles in patients with ALS and controls in various ethnic populations referring to previously reported data [15–18, 24–38]. The distributions of *ATXN2* intermediate-length CAG repeat alleles in patients with SALS and control individuals from various regions are shown in Fig. 1 and supplementary Table. As indicated in the compiled previously reported data shown in Fig. 1, the frequencies of the ALS risk alleles (CAG repeat units ≥ 29) in patients with SALS and control individuals were respectively 1.24% and 0.38% in the European and North American series [15, 16, 24–34], 0.85% and 0.12% in the Turkish series [35], 1.20% and 0.36% in the Chinese (mainland) series [36, 37], 0.77% and 0.20% in the Chinese (Taiwan) series [38], 0.97% and 0.21% in the Korean series [17], and 2.29% and 0.43% in the Brazilian (Latin American) series [18]. In contrast, the frequencies of the ALS risk alleles in patients with SALS and control individuals were respectively 0.10% and 0.20% in the Japanese series in this study. When previous studies that analyzed the frequencies of the ALS risk alleles (CAG repeat units ≥ 29) [15–18, 24–38] and the present study were subjected to a meta-analysis (14,513 cases and 18,610 controls), a high pooled OR (95% confidence interval) of 3.42 (2.73–4.29) for ALS risk alleles was obtained without evidence of significant heterogeneity ($I^2 = 0.7\%$, heterogeneity $p = 0.45$), confirming the association of the intermediate-length *ATXN2* CAG repeat alleles with increased risk for developing ALS (supplementary Figure). In this meta-analysis, OR for the Japanese population in the present study is located as a distinct outlier compared with those in other populations.

To determine whether the frequencies of large normal alleles ($24 \leq$ CAG repeat units ≤ 33) in the populations underlie the differences in the association of intermediate-length CAG repeat alleles with ALS, we compared the distributions of large normal alleles among the ethnic populations. The frequencies of large normal alleles were 2.42% in the European and North American series [15, 16, 24–34], 1.19% in the Turkish series [35], 1.64% in the Chinese (mainland) series [36, 37], 2.30% in the Chinese (Taiwan) series [38], 1.29% in the Korean series [17], and 1.92% in the Brazilian (Latin American) series [18], but only 0.92% in the Japanese series in this study. The frequency of large normal alleles was significantly higher in the European and North American series than in the Japanese series (adjusted p value = 0.006). In addition, although not statistically significant, these frequencies in the Turkish, Chinese (mainland), Chinese (Taiwan), Korean, and Brazilian (Latin American) series were also higher than that in the Japanese series.

Discussion

In this study, we demonstrated that there was no significant difference in the carrier frequency of intermediate-length CAG repeat alleles between Japanese SALS patients and controls, which is in striking contrast to previous reports showing that *ATXN2* intermediate-length CAG repeat alleles are significantly associated with the increased risk of ALS in various ethnic populations, including European and North American [15, 16, 24–34], Turkish [35], Chinese (mainland and Taiwan) [36–38], Korean [17], and Brazilian (Latin American) [18] populations. Interestingly, when we investigated the distribution of *ATXN2* large normal alleles in control individuals in other populations compiled from previously published studies, the frequency of large normal alleles was significantly higher in the European and North American series than in the Japanese series in this study. This finding is consistent with our previous study demonstrating that the frequency of intermediate-length CAG repeat alleles in *ATXN2* (≥ 23 repeat units) was significantly higher in the Caucasian series than in the Japanese series [40]. Moreover, the frequencies of large normal alleles in control individuals were also higher in the Chinese and Korean series, which geographically belong to the East-Asian series, than in the Japanese series in this study.

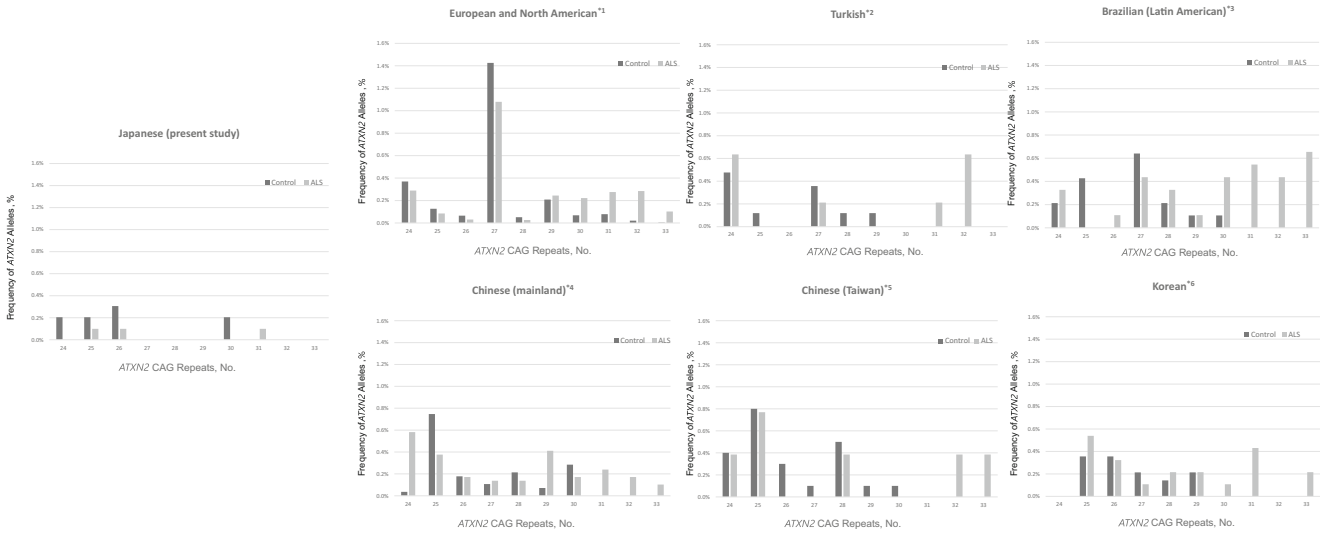

Fig. 1 Allele frequencies of *ATXN2* intermediate-length repeats in the Japanese and other populations. Distributions of *ATXN2* CAG repeats for at least 24 repeat units in patients with ALS and control series in the Japanese and other populations from various regions are shown. (*1) Included studies in European and North American series: [15, 16, 24–34]. (*2) Included study in Turkish series: [35]. (*3) Included study in Brazilian (Latin American) series: [18]. (*4) Included studies in Chinese (mainland) series: [36, 37]. (*5) Included study in Chinese (Taiwan) series: [38]. (*6) Included study in Korean series: [17].

In our previous study, we demonstrated the close associations of the relative prevalence of dominant SCAs [including SCA types 1, 2, 3 (Machado–Joseph disease), and 6, and dentatorubral-pallidoluysian atrophy (DRPLA)] in Japanese and Caucasian pedigrees with the frequencies of large normal CAG repeat alleles of the corresponding genes in these populations and suggested that large normal alleles contribute to generation of expanded alleles [40]. Regarding the prevalence of SCA2, the relative prevalence of SCA2 on a family basis was higher in Caucasian pedigrees (14%) than in Japanese pedigrees (5%) with statistically significant differences in a total of 202 Japanese and 177 Caucasian families with dominant SCA [40]. In the Korean series, it has been shown that SCA2 is the most common subtype, and the relative prevalence of SCA2 was 31.3% (10 families) in the 32 families with dominantly inherited ataxia [41, 42]. In the Taiwanese series, the prevalence of SCA2 in 81 unrelated families with autosomal dominantly inherited ataxia was 11% (nine families) [43]. These findings indicate that the relative prevalence of SCA2 is lower in Japan than in other populations. These observations raise the possibility that large normal alleles in the control populations constitute the reservoir that gives rise to intermediate-length risk alleles for SALS as well as fully expanded CAG repeats for SCA2.

Since the number of SALS cases with *ATXN2* intermediate-length CAG repeat alleles revealed in the present study was limited, it is difficult to evaluate whether there are any clinical characteristics in the ALS patients carrying *ATXN2* intermediate-length CAG repeat alleles. There is also a limitation that the ages at sampling in control subjects considerably differed from those of patients with SALS. Since the two control subjects carrying intermediate-length CAG repeat units of 30 were a 58-year-old female and a 25-year-old female at the time of sampling, the possibility of later developing neurodegenerative diseases cannot be completely excluded.

The present study demonstrated that the distribution of normal CAG repeat alleles in the Japanese population is considerably different compared with those in other populations, which seems to underlie the weak association of the intermediate-length *ATXN2* CAG repeat alleles with ALS in the Japanese population. Further investigations on regional differences in the distributions of normal *ATXN2* CAG repeat alleles among various populations will be important to better understand the association of intermediate-length CAG repeat alleles with ALS. It will also be important to investigate mechanisms of instability of CAG repeats, in particular, those associated with large normal alleles.

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Compliance with ethical standards

Genomic DNA samples were obtained from all the participants with their written informed consent, and this research was approved by the institutional review board of the University of Tokyo.

Conflict of interest The authors declare that they have no conflict of interest.

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